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Brief Clinical Report

Ring Chromosome 18 in a Fetus With Only Facial Anomalies

Frans J. Los, Cardi van den Berg, Armando P.G. Braat, Firas K. Cha'ban, Johan M. Kros, and Diane Van Opstal

Departments of Clinical Genetics (F.J.L., C. v.d. B., A.P.G.B., D.V.O.), Obstetrics & Gynecology (F.K.C.), and Pathology (J.M.K.), University Hospital Dijkzigt, Erasmus University, Rotterdam, The Netherlands

We report on a prenatally detected case of ring chromosome 18 [46,XX,r(18)] in amniotic fluid cells of a fetus with an abnormal facial profile on ultrasound as the only malformation. The chromosome 18 origin of the ring chromosome, of a supernumerary marker chromosome in some cells, and of micronuclei was demonstrated by fluorescent in situ hybridization with a whole chromosome 18 paint (Cambio) and 18 centromere probe L1.84. DNA investigations showed deletions of 18p as well as 18q material of r(18), which turned out to be of paternal origin. Autopsy of the fetus after termination of pregnancy at 20 weeks of gestation showed no additional malformations, in agreement with the previous ultrasound findings.

KEY WORDS: ring chromosome 18, amniotic fluid cells, ultrasound, facial dysmorphisms

INTRODUCTION

Some cases of prenatally detected structural chromosome 18 abnormalities have been reported: isochromosome 18q [i(18q)] [Froster-Iskenius et al., 1984; Wurster-Hill et al., 1991], mosaic i(18p) [Göcke et al., 1986], 18p- [Göcke et al., 1988], mosaicism of deletion (18)(p11)/i(18q) [Sutton and Ridler, 1986], and ring chromosome 18 [r(18)] [Eiben et al., 1992]. We describe the prenatal detection of a 46,XX,r(18) karyotype in amniotic fluid cells investigated with conventional cytogenetic techniques and fluorescent in situ hybridization (FISH). DNA investigations for the establishment of the parent of origin as well as potential deletions of 18p and 18q material of the r(18) were carried out. Furthermore, the prenatal ultrasound findings and a detailed clinical description are presented.

CLINICAL REPORT

A 39-year-old pregnant woman (G4, P2, Ab1) asked for prenatal diagnosis because of advanced maternal age. In 2 previous pregnancies, prenatal diagnosis had also been performed with normal results and favourable pregnancy outcome. She had a sister with Down syndrome; the family history of her husband (40 years old) was unremarkable. Amniocentesis was performed at a gestational age of 16 weeks. No abnormalities were noted on ultrasound investigation. After the finding of a r(18), detailed ultrasonography was performed at 19 weeks of gestation; no structural malformations were seen in this fetus with normal biometric values (Table I). Intracranial anatomy was normal. The only remarkable finding was a slightly abnormal facial profile with a receding forehead, pronounced upper lip/philtrum and rethrognathia (Fig. 1), which might fit the fetal phenotype of the 18p-syndrome [Göcke et al., 1988]. Since the r(18) turned out to have risen de novo and the chances for a normal phenotype were counselled low [Schinzel, 1984], the parents opted for termination of pregnancy. At 20 weeks of gestation, labour was induced by intravenously administered prostaglandin. A female fetus was born weighing 270 g (mean-1× S.D.) [Chambers et al., 1993] with normal body measurements (Table I). A receding forehead, pronounced convex philtrum, micro- and rethrognathia, a broad neck, hypoplastic alae nasi, and abnormally modeled ears were noted (Fig. 2). Autopsy did not demonstrate any internal malformation; all organs showed a normal weight and development for gestational age. The brain had developed normally with normal midline structures.

CYTOGENETIC AND DNA STUDIES

Amniotic fluid cells were cultured by the in situ method on glass coverslips. Trypsin-Giemsa staining was used. The karyotype was 46,XX,r(18) in much investigated clones (21 out of 27). In 5 clones mosaicism of 45,XX,-18/46,XX,r(18) was encountered and one clone showed a mosaicism of 45,XX,-18/ 46,XX,r(18)/

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Address reprint requests to Dr. Frans J. Los, Department of Clinical Genetics, University Hospital Dijkzigt, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

TABLE I. Fetal Ultrasonographical Biometry at 19 Weeks and Body Measurements After Termination of Pregnancy at 20 Weeks of the Fetus With 46,XX,r(18)*

Parameter[a]	Ultrasound biometry				Body measurements	
	Fetus (mm)	Reference values (mm) 5%	50%	95%	Fetus (mm)	Reference values (mm) mean ± 1 S.D.
CHL	—	—	—	—	248	261 ± 17
HC	151	141	152	164	170	175 ± 12
DBP	41	39	43	47	—	— —
IOD/ICD	11.5	8	11	14	12	13.6 ± 1.8
OOD/OCD	27.2	26	30	34	34	35.9 ± 3.4
Femur length	27	24	27	30	—	— —
AC	133	114	128	144	—	— —
Hand length	—	—	—	—	24	29.5 ± 2.9
Middle finger length	—	—	—	—	10	12.2 ± 1.5
Foot length	—	—	—	—	29[b]	35 ± 2.5
IND	—	—	—	—	35	34.8 ± 3.4

*Ultrasonographical reference values according to Snijders and Nicolaides [1994] and Trout et al. [1994]; body measurement reference values according to Chambers et al. [1993].
[a]CHL, crown to heel length; HC, head circumference; DBP, distantia biparietalis; IOD/ICD, inter orbital distance/inner canthal distance; OOD/OCD, outer orbital distance/outer canthal distance; AC, abdominal circumference; IND, inter nipple distance.
[b]Measurement outside mean ± 2 S.D. area.

47,XX,r(18),+ marker chromosome (Fig. 3). In one cell, a double-size (dicentric) ring chromosome was found (Fig. 3). In fetal fibroblasts, only the 46,XX,r(18) line was found in 16 investigated cells. Karyotypes of the parents were normal 46,XY and 46,XX, respectively.

FISH was performed on unstained slides of cultured amniotic fluid cells with a whole chromosome 18 paint (Cambio Ltd., Cambridge, U.K.) and chromosome 18 centromere probe L1.84 [Devilee et al., 1986]. Hybridization with the chromosome 18 paint was performed according to the procedure recommended by the manufacturer. FISH with L1.84 was done as described before [Van Opstal et al., 1995]. Slides were examined with a Leitz Aristoplan fluorescence microscope and images were captured by the Genetiscan Probe Master System (Perceptive Scientific Instruments Ltd., Chester, U.K.). Hybridization with the whole chromosome 18 paint resulted in fluorescent staining of both

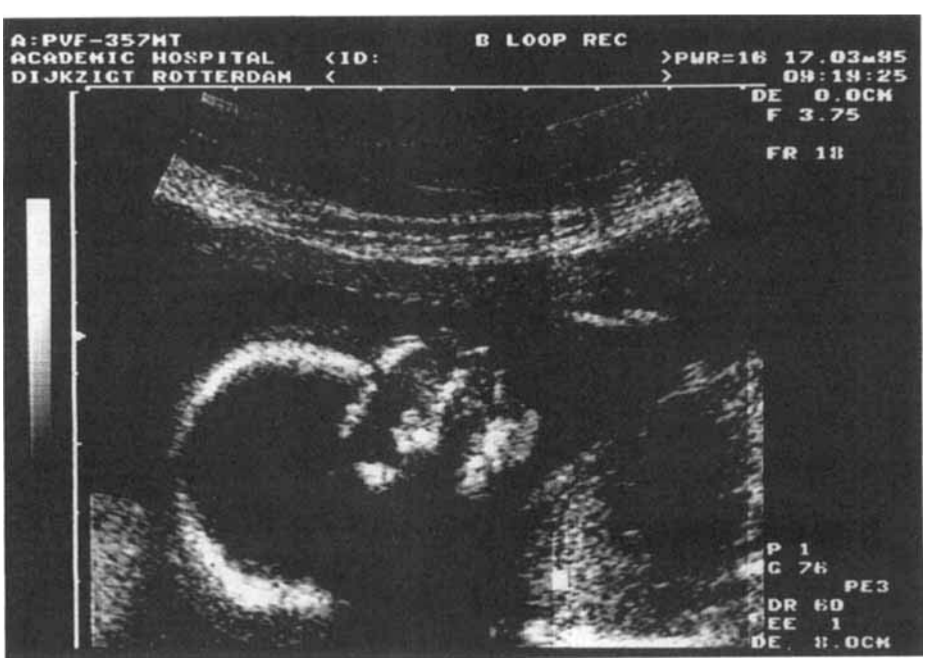

Fig. 1. Ultrasonographical image of the fetal facial profile at 19 weeks, showing the receding forehead, rethrognathia and pronounced upper lip/philtrum.

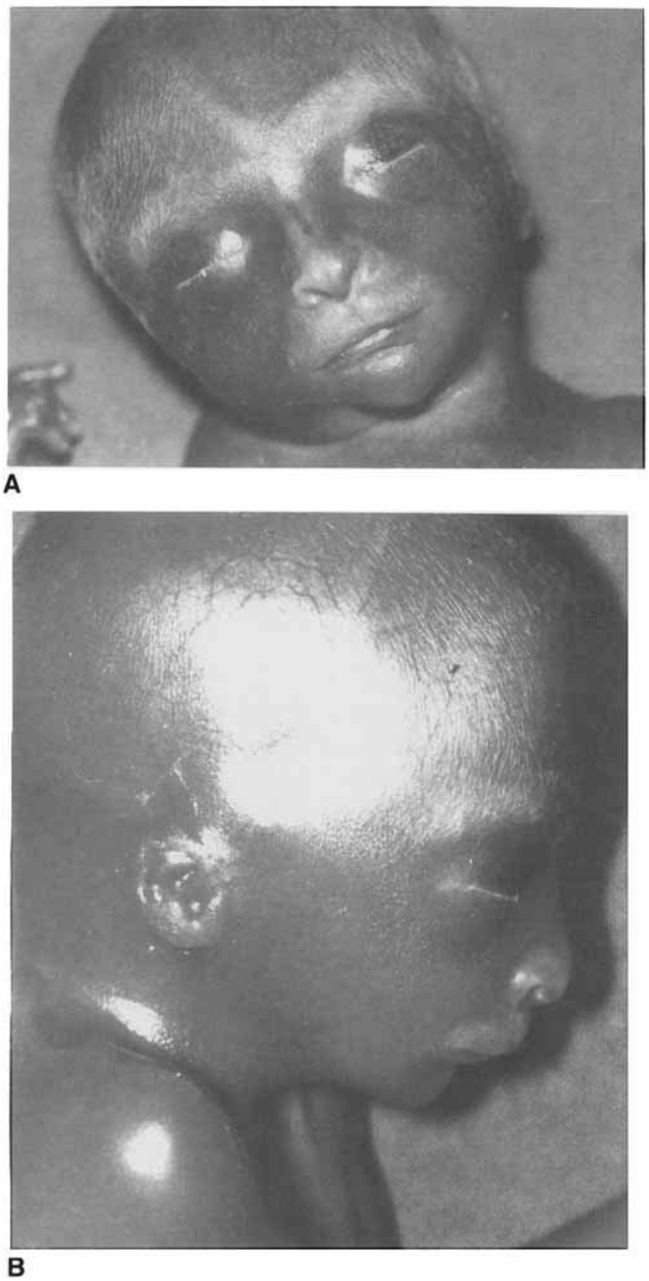

Fig. 2. **A**: Frontal and (**B**) lateral view of the fetus at 20 weeks, showing the receding forehead, hypoplastic alae nasi, pronounced convex philtrum and micro/rethrognathia.

ring and marker chromosome (Fig. 4A), as well as of micronuclei found in the vicinity of some interphase nuclei, indicating a chromosome 18 origin. Hybridization with L1.84 yielded strong signals on both ring and marker chromosome (Fig. 4B), also demonstrating a chromosome 18 origin.

DNA isolated from cultured amniotic fluid cells and blood of both parents was investigated by performing polymerase chain reaction (PCR) analysis of various microsatellite markers on chromosome 18 to establish potential deletions of 18p and 18q material of r(18) and determine the parent of origin (Fig. 5). The PCR products of D18S59 and D18S40, located on 18p, and of D18S34, D18S35, D18S42, MBP and D18S70, located on 18q [Le Beau et al., 1993; Geurts van Kessel et al., 1994] showed an informative pattern. Unfortunately, 18p marker D18S52 and 18q marker D18S38 [Le Beau et al., 1993; Geurts van Kessel et al., 1994] turned out to be non-informative. PCR analysis demonstrated the absence of the paternal alleles in fetal cells at the loci D18S59, D18S70, MBP, and D18S42 whilst a paternal as well as a maternal allele were present at the loci D18S35, D18S34, and D18S40 (Fig. 5). These DNA investigations indicated that r(18) was of paternal origin and displayed an 18p deletion of undetermined size together with a large 18q deletion, at least del(18)(q21.33).

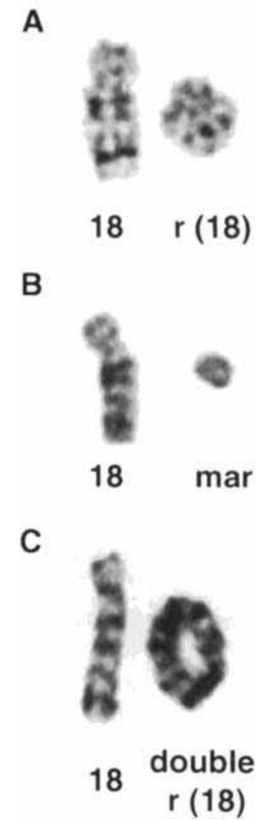

Fig. 3. Partial karyotype of cultured amniotic fluid cells (Trypsin-Giemsa staining). **A**: r(18). **B**: Marker chromosome (mar). **C**: Double r(18), each accompanied by the normal chromosome 18.

DISCUSSION

A de novo r(18) was established prenatally in amniotic fluid cells with cytogenetic evidence for mitotic instability such as intraclonal mosaicism [Rocchi et al., 1984; Kosztolányi, 1987a], the presence of a supernumerary marker chromosome [Koulischer et al., 1980; MacDermot et al., 1990], dicentric ring formation [Kosztolányi, 1987a, b], and the occurrence of micronuclei [Kosztolányi, 1987a]. FISH showed the ring and marker chromosome to be chromosome 18-derived and the micronuclei to contain chromosome 18 material.

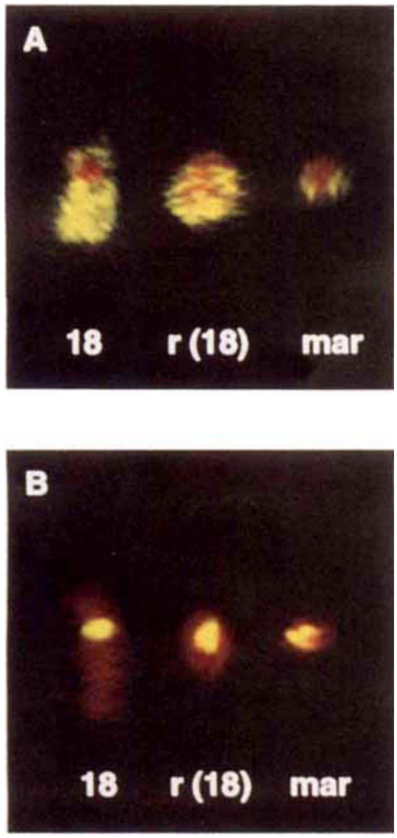

Fig. 4. FISH signals on normal chromosome 18, r(18) and marker chromosome (mar) after in situ hybridization with (**A**) whole chromosome 18 paint (Cambio) and (**B**) 18 centromere probe L1.84 to cultured amniotic fluid cell metaphases.

DNA investigations demonstrated a deletion of 18p and 18q material of r(18), which was of paternal origin. These investigations were performed on DNA isolated from amniotic fluid cells after termination of pregnancy, but they could have been performed prenatally. Especially in cases of de novo ring chromosomes without any ultrasound abnormality, in which the fetus might only be affected with the "ring syndrome" [Kosztolányi, 1987b; Pezzolo et al., 1993], DNA data concerning subtelomeric deletions are important for genetic counselling.

In contrast to the expected concomitance of 18p and 18q deletions with serious fetal malformations, the ultrasound findings were surprisingly normal, apart from the facial profile. This case confirms the importance of the interpretation of fetal facial profile abnormalities on ultrasound, which turned out to be in agreement with clinical observations of the fetus after termination of pregnancy.

The fetus had no malformations of the internal organs, but only facial anomalies, which is in contrast with an earlier prenatal diagnosis of de novo r(18) [Eiben et al., 1992]. However, the facial anomalies resembled those of a reported prenatal case with 18p- syndrome [Göcke et al., 1988], and, remarkably, also those of cases with tetrasomy 18p [Göcke et al., 1986] and mosaic monosomy 18p/trisomy 18q [Sutton and Ridler, 1986]. The hypoplastic alae nasi, together with some other reported anomalies of the 18p-/r(18) phenotype such as absent permanent teeth, hypothyroidism, diabetes mellitus and anorectal malformations [Schinzel, 1984], suggest similarities between this phenotype and the autosomal recessive inherited Johanson Blizzard syndrome (JBS)[phenotype 243800; McKusick, 1994]. Some cases of JBS might be recurrences of chromosome 18 microdeletions, transmitted through parental germ line mosaicism, a well-known alternative explanation for autosomal recessive inheritance [Petrella et al., 1993]. The phenotypic overlap between the r(18)/18p-syndrome and JBS leads to the assumption that the 18p region is a candidate area for a potential JBS gene.

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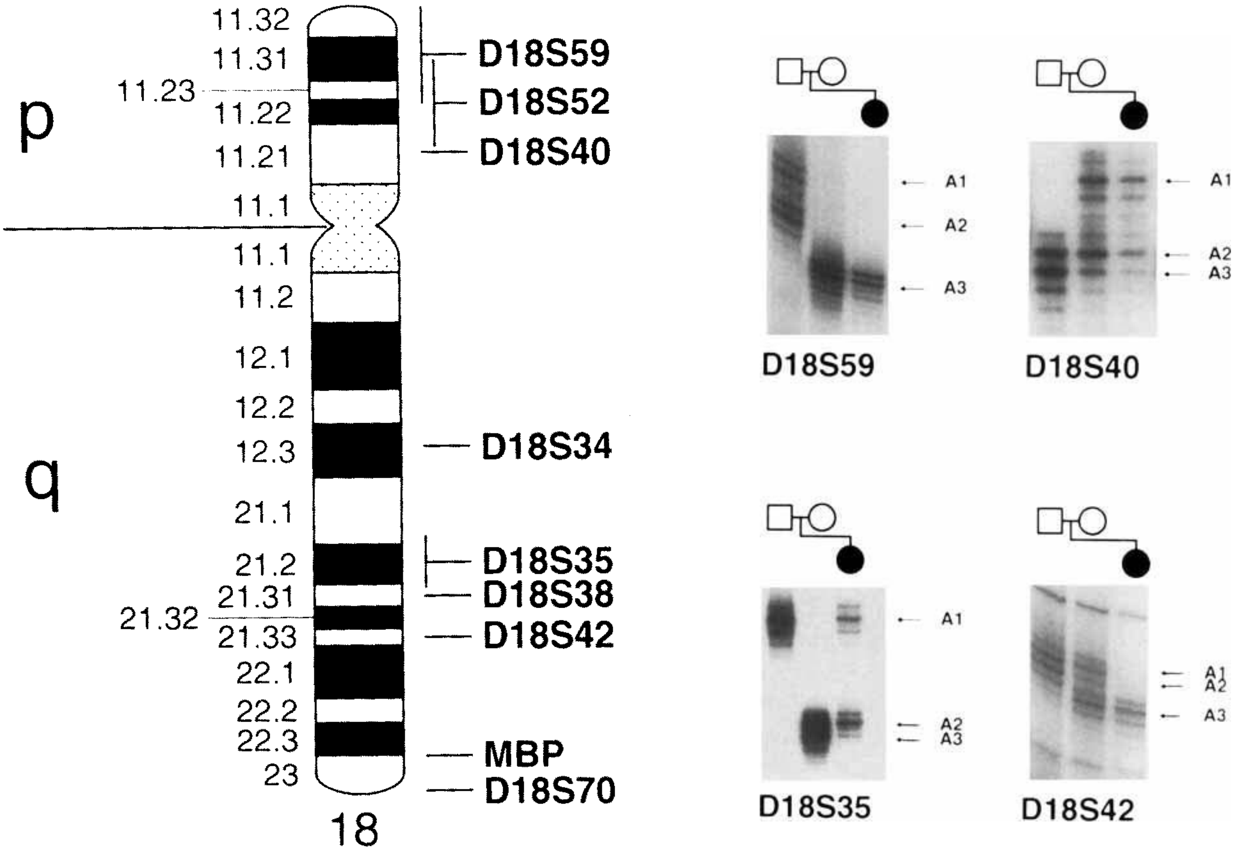

Fig. 5. Ideogram of chromosome 18 with the localization of the tested microsatellite markers. PCR analysis of D18S59, D18S40, D18S35 and D18S42 shows the absence of paternal alleles A_1 or A_2 at loci D18S59 and D18S42 in fetal cells.

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